SEROTAXONOMY OF *SOLANUM, CAPSICUM, DUNALIA,* AND OTHER SELECTED SOLANACEAE[1,2]

RICHARD N. LESTER[3] AND PHILIP A. ROBERTS[4]

ABSTRACT

Serotaxonomic comparisons were made on about 50 species of Solanaceae. Immunoabsorption data were obtained from reactions of seed protein antigen systems with antibody systems absorbed by several antigen systems in numerous permutations. Relationships of the taxa were computed by newly developed methods. Twenty species of *Solanum,* mostly of subg. *Leptostemonum,* were compared using six antisera. *Solanum nigrum* (subg. *Solanum*) was serologically distinct from the others. Several groups of species belonging to different sections of *Solanum* were recognized. Phenetic and phyletic relationships within sect. *Androceras* were explored. Re-analysis of a complete cubic matrix of immunoabsorption data for eight species of *Solanum* rearranged relationships slightly but emphasized the divergence between different sections of this genus. A broad survey was made using an antibody system raised to *Capsicum annuum.* This revealed the distinctiveness of *Nicandra* from other Solanaceae, several possible inter-generic relationships, but also some unlikely ones. Further studies revealed closer relationships of *Dunalia* species to *Capsicum* than to *Iochroma.*

Protein characters are valuable in plant taxonomy for assessing inter-generic and even inter-family relationships (Jensen & Fairbrothers, 1983). Within the Solanaceae several serological studies have been made by workers such as Chester, Hammond, Tucker, Gray, and Lester (see review by Lester, 1979).

Because of the complex physico-chemical properties of proteins, and the even more complex phenomena of immunological reactions, many different serotaxonomic techniques have been developed (Jensen & Fairbrothers, 1983). Immunoabsorption resolves subsets of antigenic determinant sites as recognized by corresponding subsets of antibodies after the removal of common antibodies from the antibody system by absorption by other antigen systems. Recent developments in the theoretical and practical aspects of immunoabsorption techniques and the analysis of the resultant data by appropriate numerical taxonomic procedures have been discussed in detail (Lester, 1979; Lester et al., 1983). For this paper these procedures were applied to selected species of Solanaceae.

MATERIALS AND METHODS

Seed samples from the Birmingham University Solanaceae Collection (Tables 1–3) were used to prepare protein extracts, to induce antibody production in rabbits, and for subsequent immunological experiments using absorbed antibody systems. Procedures, especially the scoring and analysis of results, followed those described by Lester (1979) and Lester et al. (1983).

Three sets of experiments were conducted.

1. Twenty accessions of *Solanum,* mostly of subg. *Leptostemonum* (Table 1) were compared using six antibody systems (data published in Lester et al., 1983).
2. Eight species from diverse sections of the genus *Solanum* (Table 2) were compared using eight antibody systems (data published in Lester, 1979).
3. About 25 species of *Capsicum, Solanum,* and other genera of Solanaceae, mostly of tribe Solaneae (Table 3) were compared using one antibody system (Lester, unpubl. data).

Most of these data have now been analyzed by most of the procedures described by Lester et al. (1983), especially by using Jaccard's and similar coefficients to estimate phenetic relationships. Some of the data sets also were subjected to cladistic analysis. Many dendrograms were produced by different analyses of the various sets and subsets of data, some of which are presented herein (Figs. 1–6).

[1] We are grateful to Sarah Marsh for growing these plants, to the Science and Engineering Research Council (U.K.) for financial support, and to Julie Brean, Barbara Klauza, and Sarah Sutton for technical assistance.
[2] This paper was part of the Second International Symposium of the Biology and Systematics of the Solanaceae presented at the Missouri Botanical Garden on 3–6 August 1982.
[3] Department of Plant Biology, University of Birmingham, Edgbaston, Birmingham B15 2TT, England.
[4] Department of Nematology, University of California, Riverside, California 92521.

TABLE 1. Listing and classification of the 20 accessions of *Solanum* species that were compared immunologically using antibody systems to CAP, ROS, SIS, QUT, TOR, and PRN.

Taxa	Code	Acc. No.
Subg. *Leptostemonum* (Dun.) Bitt.		
Sect. *Acanthophora* Dun.		
S. capsicoides All.	CAP	S.0866
S. chloropetalum Schl.	CHL	S.0021
S. viarum Dun.	VIA	S.1418
Sect. *Androceras* (Nutt.) Bitt. ex M.		
Ser. *Androceras*		
S. rostratum Dun.	ROS	S.0097
	ROS	S.0399
S. fructo-tecto Cav.	FTO	S.0025
Ser. *Violaceiflorum* Whalen		
S. heterodoxum Dun.	HET	S.0593
S. citrullifolium A. Br.	CIT	S.0195
	CIT	S.0127
Sect. *Cryptocarpum* Dun.		
S. sisymbriifolium Lam.	SIS	S.1099
	SIS	S.0136
Sect. *Lasiocarpa* (Dun.) D'Arcy		
S. hirtum Vahl	HIR	S.1142
S. quitoense Lam.	QUT	S.0972
S. tequilense A. Gray	TEQ	S.0973
Sect. *Oliganthes* (Dun.) Bitt.		
S. anguivi Lam.	ANG	S.1335
S. prinophyllum Dun.	PRN	S.0386
	PRN	S.1444
Sect. *Torva* Nees		
S. hispidum Pers.	HIS	S.0017
S. torvum Swartz	TOR	S.0839
Subg. *Solanum*		
Sect. *Solanum*		
S. nigrum L.	NIG	S.0498

TABLE 3. List of accessions of *Capsicum*, *Dunalia*, and other genera of Solanaceae that were compared immunologically using antibody system to *Capsicum annuum*.

Code	Acc. No.	Taxa
ATHEN PIC	S.1069	*Athenaea picta*
ATROP BEL	S.0077	*Atropa belladonna*
CAPSI ANN	S.1083	*Capsicum annuum*
CAPSI BAC	S.0749	*C. baccatum*
CAPSI CHA	S.0750	*C. chacoense*
CYPHO BET	S.0045	*Cyphomandra betacea*
DATUR STR	S.0185	*Datura stramonium*
DUNAL AUS	S.0379	*Dunalia australis*
DUNAL BRE	S.0375	*D. breviflora*
DUNAL BRF	S.0377	*D. breviflora*
DUNAL LOR	S.0376	*D. lorentzii*
DUNAL TUB	S.1094	*D. tubulosa*
HYOSC NIG	S.0289	*Hyoscyamus niger*
IOCHR SPE	S.1599	*Iochroma* sp.
IOCHR UMB	S.1602	*I. umbrosa*
LYCIU CES	S.0368	*Lycium cestroides*
LYCOP ESC	S.1152	*Lycopersicon esculentum*
NICAN PHY	S.0082	*Nicandra physalodes*
NICOT TAB	S.0329	*Nicotiana tabacum*
PHYSA ANG	S.0512	*Physalis angulata*
SALPI ORI	S.0159	*Salpichroa origanifolia*
SARAC UMB	S.0117	*Saracha umbellata*
SCOPO LUR	S.0104	*Scopolia lurida*
SOLAN AET	S.0156	*Solanum aethiopicum*
SOLAN CAP	S.0263	*S. capsicastrum*
TRECH SAT	S.0234	*Trechonaetes sativa*
WITHA SOM	S.0242	*Withania somnifera*
WITHE COC	S.1582	*Witheringia coccoloboides*

TABLE 2. List of eight species of *Solanum* that were compared immunologically using antibody systems to all eight species.

Code	Acc. No.	Species	Section
TUBERO	S.0952	*S. tuberosum* L.	*Petota*
SCABRU	S.0243	*S. scabrum* Mill.	*Solanum*
MAURIT	S.0049	*S. mauritianum* Scop.	*Brevantherum*
HENDER	S.0167	*S. ×hendersonii* Hort.	*Pseudocapsicum*
SEAFOR	S.0051	*S. seaforthianum* Andr.	*Jasminosolanum*
AETHIO	S.0279	*S. aethiopicum* L.	*Oliganthes*
SIMILE	S.0211	*S. simile* F. Muell.	*Archaesolanum*
CITRUL	S.0168	*S. citrullifolium* A. Br.	*Androceras*

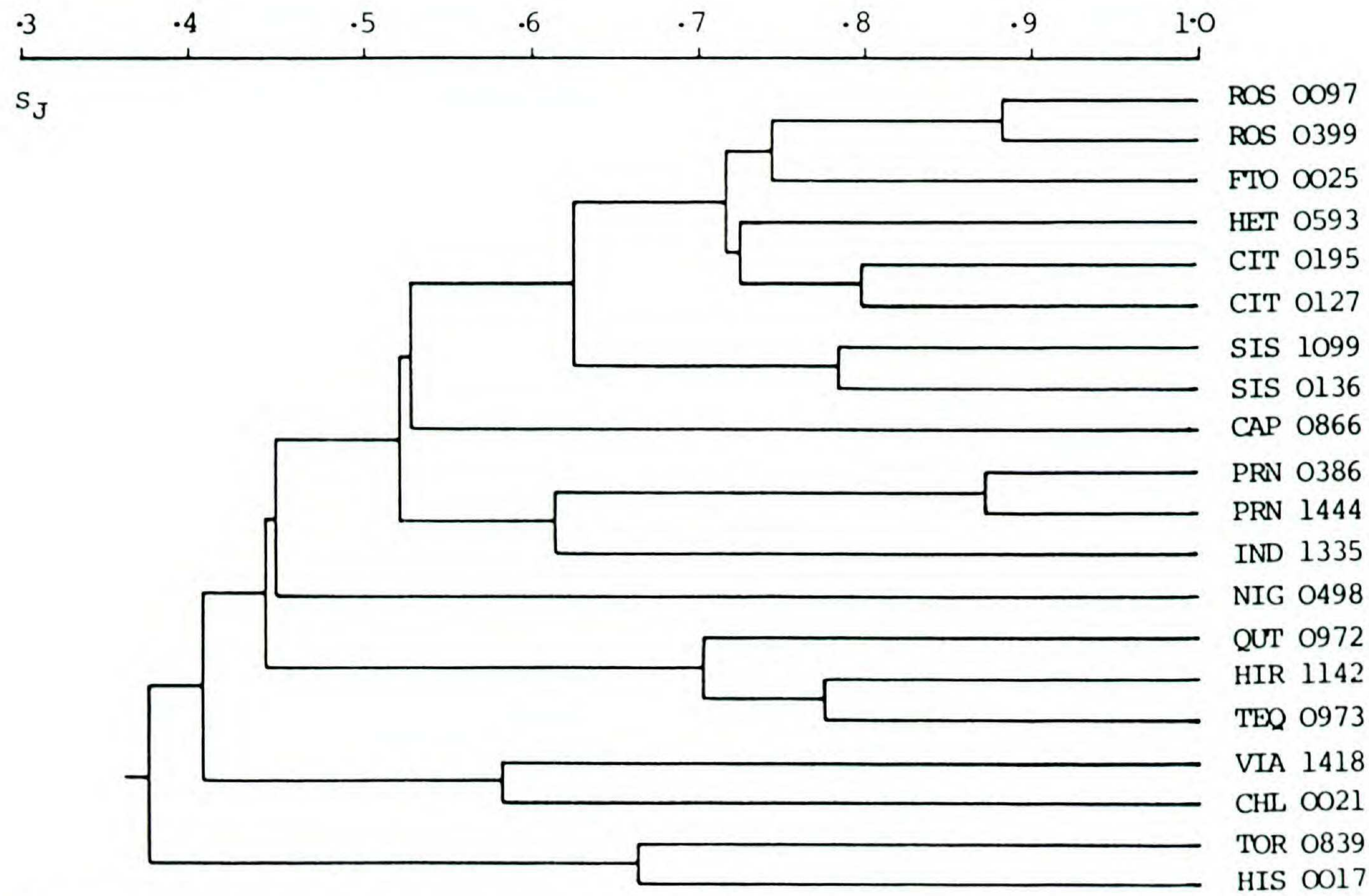

FIGURE 1. Phenogram of immunological similarities of 20 accessions of *Solanum* species calculated by Jaccard's coefficient and group average clustering (for explanation see text and Table 1).

RESULTS AND DISCUSSION

SOLANUM SUBG. *LEPTOSTEMONUM*

This set of data has already been described and analyzed in several ways (Lester et al., 1983): Jaccard's coefficient and group average clustering is justified on theoretical grounds and produces a taxonomically acceptable phenogram (Fig. 1).

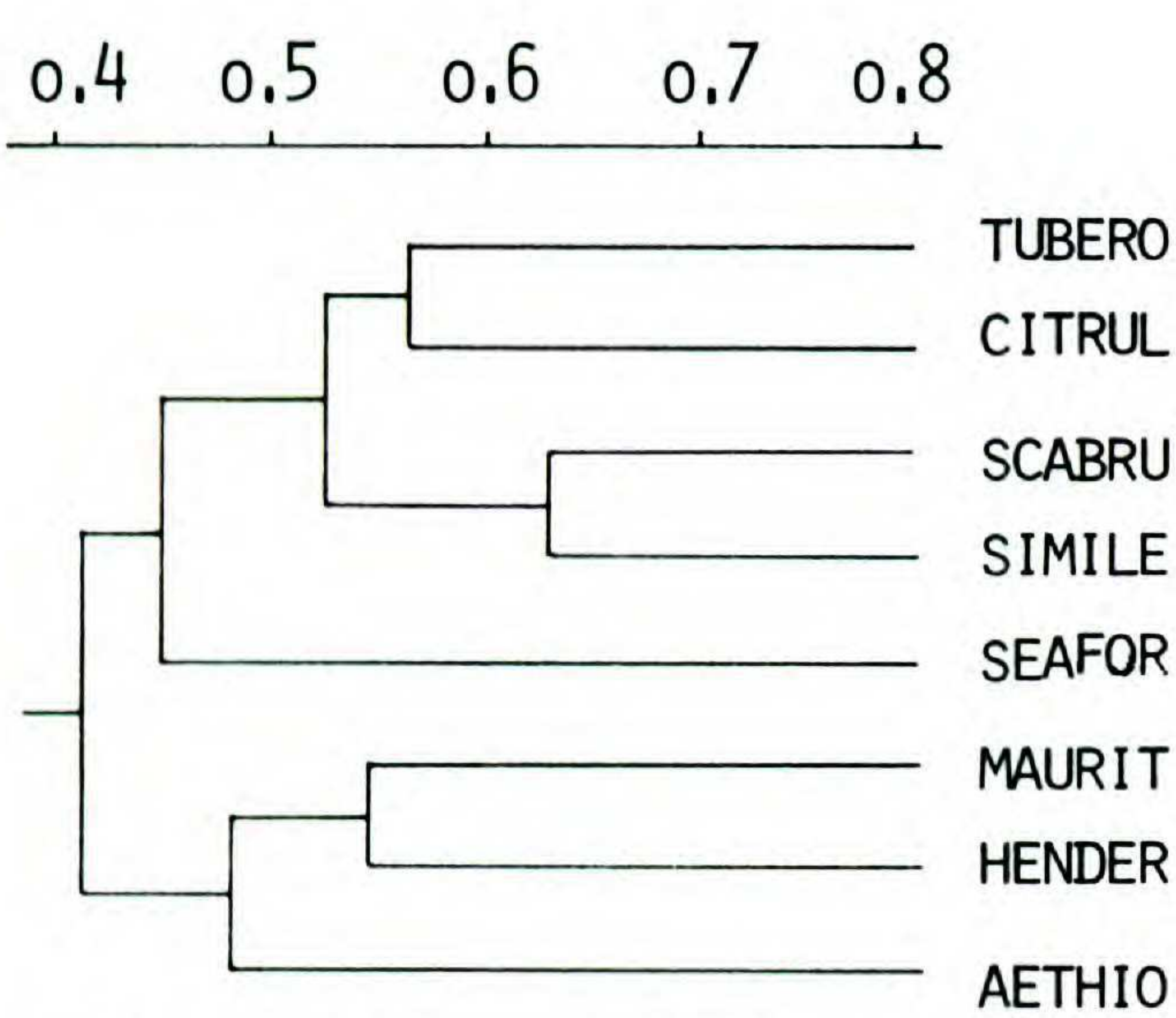

FIGURE 2. Phenogram of immunological similarities of eight *Solanum* species calculated by Jaccard's coefficient and group average clustering (for explanation see text and Table 2).

Solanum nigrum showed little similarity to the other species, which supports the major taxonomic distinction between subg. *Solanum* and subg. *Leptostemonum*. *Solanum torvum* and *S. hispidum* of sect. *Torvaria* were grouped together but were well separated from any other taxa. *Solanum hirsutum, S. tequilense,* and *S. quitoense,* members of the distinctive sect. *Lasiocarpa,* were grouped together and separated from other taxa. *Solanum chloropetalum* and *S. viarum,* two morphologically similar and partly interfertile species of sect. *Acanthophora,* were grouped together. *Solanum capsicoides,* of the same section, was placed some distance away, but the antigen system of this species produced nonspecific reactions.

The two accessions of *Solanum prinophyllum* from Australia were placed together and were joined at a low level by *S. anguivi* from Africa, which is also classified in sect. *Oliganthes.*

The two accessions of *Solanum sisymbriifolium,* sect. *Protocryptocarpum,* were placed together and were linked with members of sect. *Androceras.* Two accessions of *S. citrullifolium* were joined by *S. heterodoxum,* also of ser. *Violaceiflorum,* and two accessions of *S. rostratum* were joined by *S. fructo-tecto,* also of ser. *Androceras.*

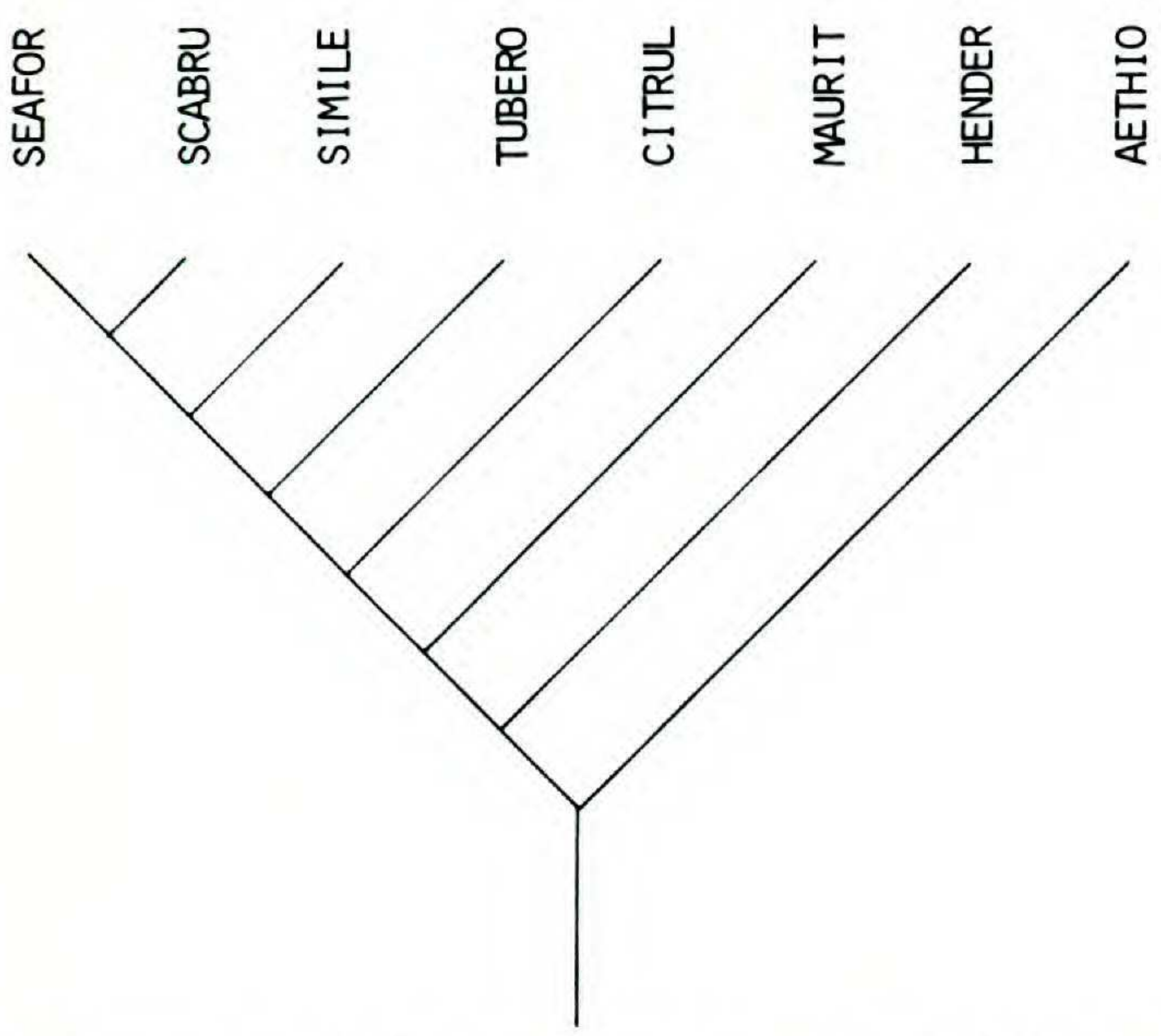

FIGURE 3. Cladogram of eight species of *Solanum* derived from immunological data by the Dollo method (for explanation see text and Table 2).

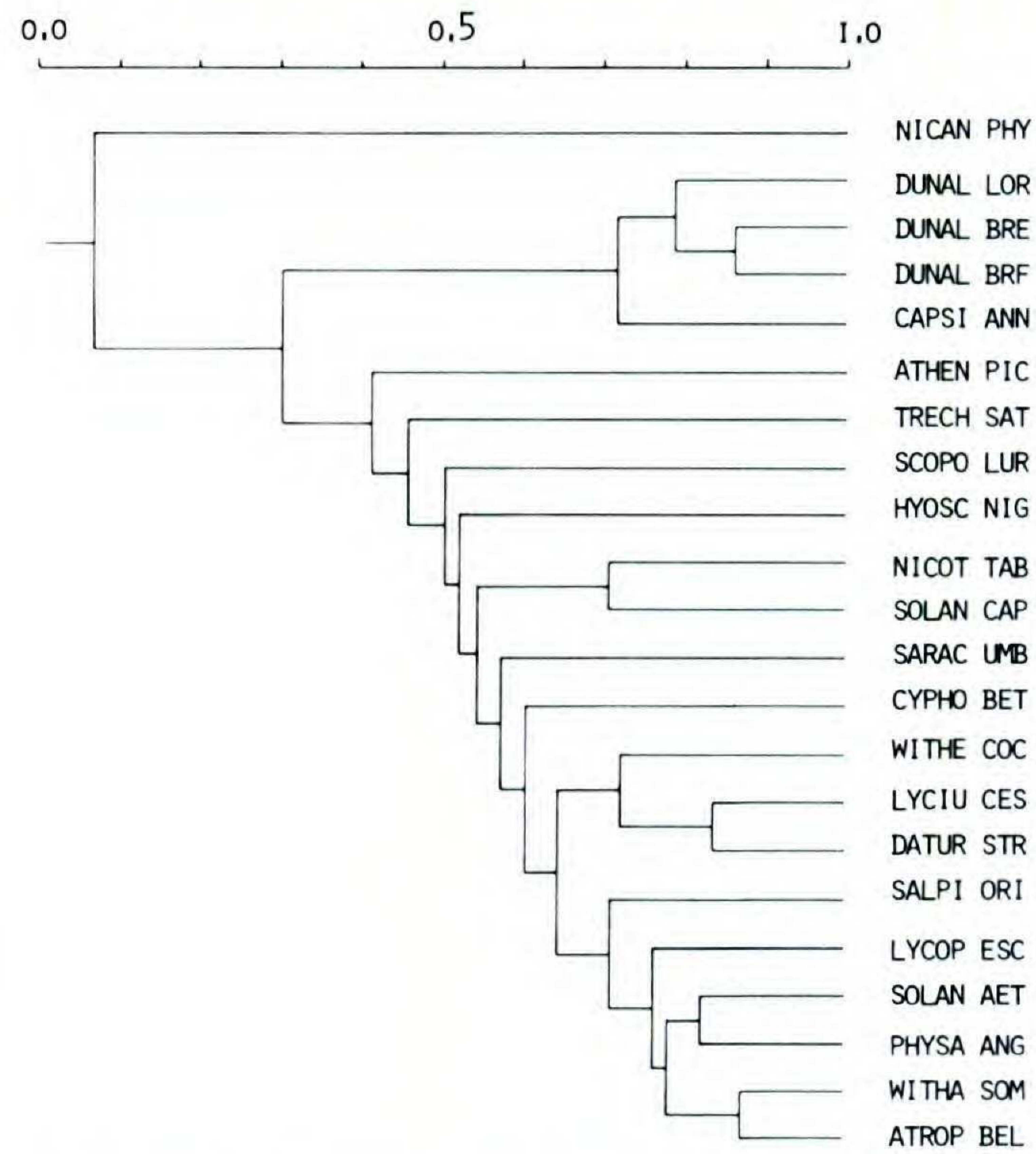

FIGURE 4. Phenogram of immunological similarities of 22 accessions of various genera of Solanaceae calculated by Jaccard's coefficient and group average clustering (for explanation see text and Table 3).

Nearly all of these serologically indicated relationships made good taxonomic sense, but in most cases the various sections of *Solanum* are very distinct and there is little information on the affinities between them.

An essay at cladistic analysis (Lester et al., 1983) using only data from the antibody system to *S. rostratum* suggested an evolutionary sequence (with distances between evolutionary units as indicated in parentheses): ancestor (17), SIS S.0136, SIS S.1099, (6) HET S.0593 (3) CIT S.0127, (3) CIT S.0195, (5) FTO S.0025 (4) ROS S.0097, ROS S.0399. This is a progression from *S. sisymbriifolium,* a perennial plant with actinomorphic violet flowers and red berries, through to *S. rostratum,* a normally short-lived annual plant with strongly zygomorphic yellow flowers and dry capsules. In each morphological attribute an evolutionary sequence can be recognized going from *S. sisymbriifolium,* through *S. heterodoxum, S. citrullifolium* and *S. fructo-tecto* to *S. rostratum.* These conclusions are the converse of Whalen's (1979) evolutionary scheme, but are supported by spermoderm studies (Lester, unpubl. data).

Other cladograms, produced from these immuno-absorption data, were published by Lester et al. (1983). One cladogram had successive simple branches to *S. quitoense, S. torvum, S. capsicoides, S. prinophyllum,* and finally *S. sisymbriifolium* and *S. rostratum.*

Cladistic analysis of the total data set was made by computer using the Dollo and Wagner methods (Felsenstein, 1982), but the results are not presented here because the data were analyzed in only one sequence and the resultant cladograms were not taxonomically acceptable.

EIGHT DIVERSE SECTIONS OF *SOLANUM*

This set of data was described and analyzed in an elementary way in Lester (1979). Re-analysis, using the preferred Jaccard's coefficient and group average clustering, provided a new dendrogram (Fig. 2).

Some taxonomic groupings are maintained, such as those of *Solanum scabrum* and *S. simile* (sects. *Solanum* and *Archaesolanum*) and *S. hendersoni, S. mauritianum,* and *S. aethiopicum* (sects. *Pseudocapsicum, Brevantherum,* and *Oliganthes*), but *S. citrullifolium* (sect. *Androceras*) is now grouped with *S. tuberosum* (sect. *Petota*), which is unacceptable on morphological grounds. *Solanum seaforthianum* (sect. *Jasminosolanum*) links with the rest at a very low level. The taxonomic relationships between these sections have been discussed previously (Lester, 1979). In general these results suggest that most of these sections of the genus *Solanum* are diverse and are not closely related to each other. In such a situation, different procedures of analysis can produce radically different dendrograms. The inclusion of many more species, even without using any more antibody systems, would improve the taxonomic information and the stability of the classifications.

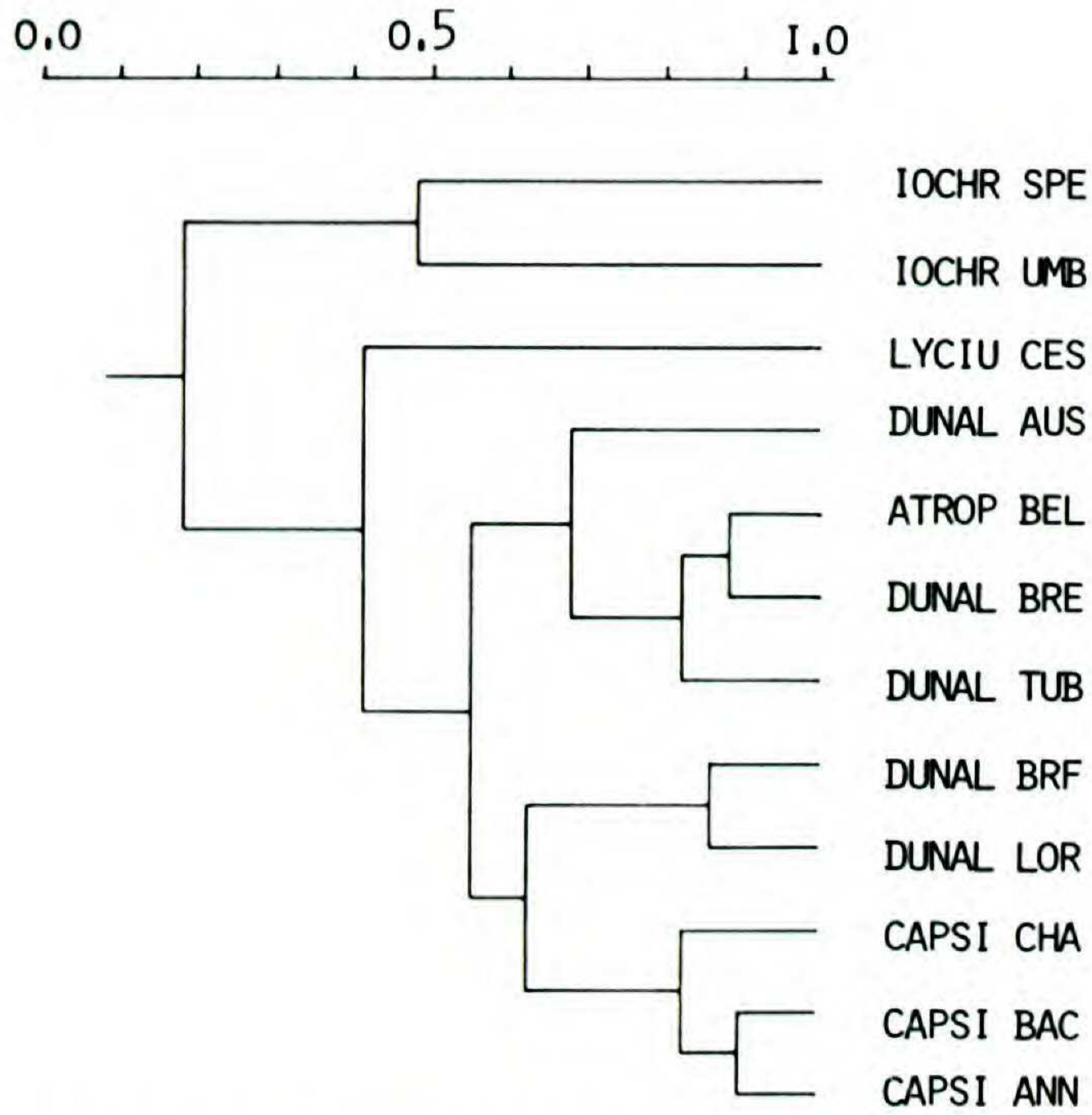

FIGURE 5. Phenogram of immunological similarities of 12 accessions of *Capsicum*, *Dunalia*, and other genera calculated by similarity ratio (coeff. no. 28) and group average clustering (for explanation see text and Table 3).

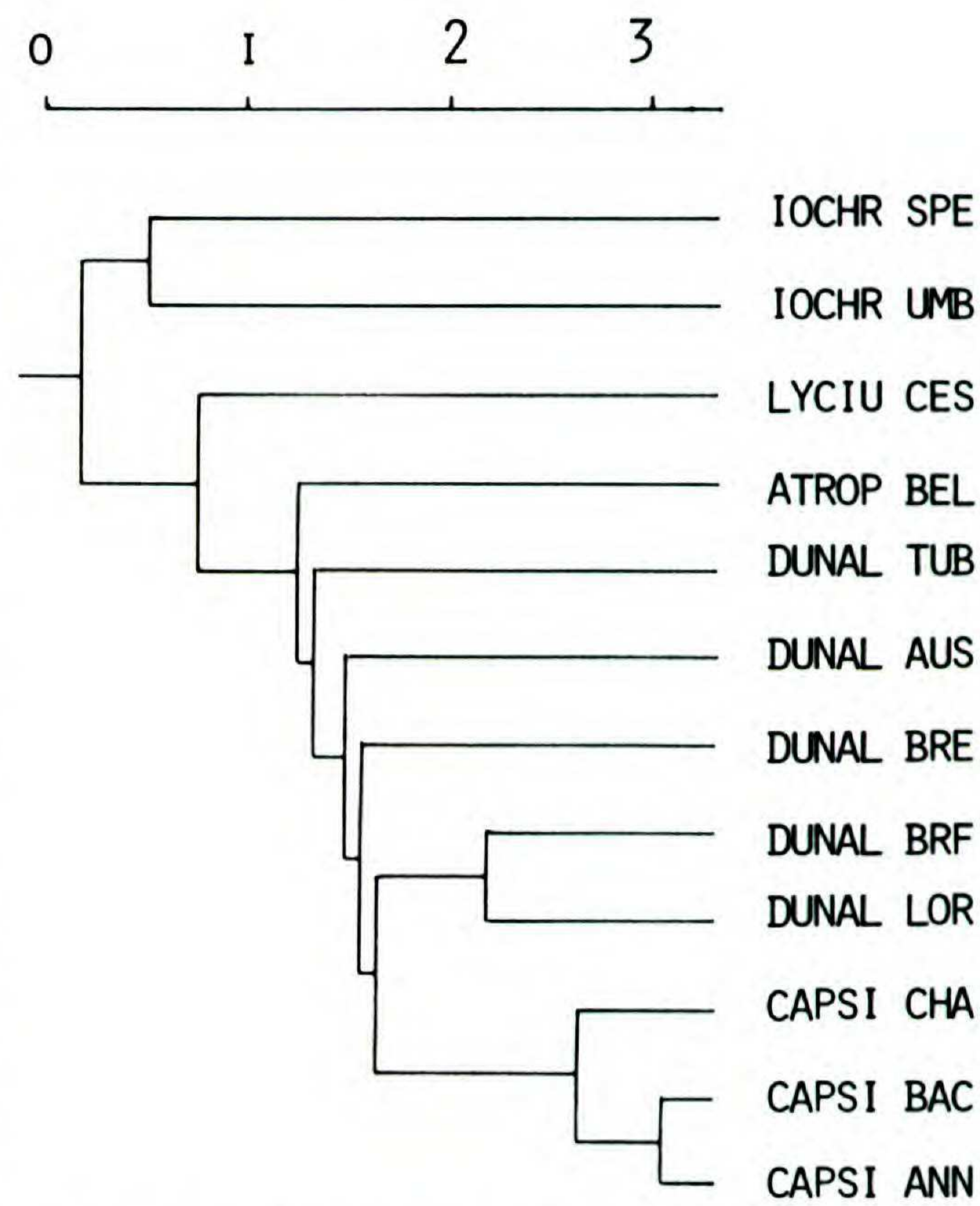

FIGURE 6. Phenogram of immunological similarities of 12 accessions of *Capsicum*, *Dunalia*, and other genera calculated by dot product and furthest neighbour clustering (for explanation see text and Table 3).

Cladistic analysis using the Dollo method on Felsenstein's Phylogeny Inference Package (PHYLIP version 2.3) (Felsenstein, 1982) produced a rooted tree suggesting an evolutionary sequence with separate branches to *S. aethiopicum*, *S. hendersonii*, *S. mauritianum*, *S. citrullifolium*, *S. tuberosum*, *S. simile*, and *S. seaforthianum* (Fig. 3).

It is interesting that the three representatives of subg. *Leptostemonum*, a group with long thin anthers, stellate hairs, and often with prickles, appear to be more ancestral, whereas four representatives of subg. *Solanum* and *Archaesolanum* have a more derived status. This suggestion that subg. *Leptostemonum* is ancestral and that subg. *Solanum* is derived is controversial.

Cladistic analysis by the Wagner method (Felsenstein, 1982) produced an unrooted tree with the species in the same sequence as the Dollo method, but this sequence could be read in either direction.

CAPSICUM AND OTHER GENERA

These were preliminary experiments using antiserum to only *Capsicum annuum*.

A study of 19 genera, mostly of the tribe Solaneae, (Fig. 4) showed the distinctiveness of *Nicandra* from the other genera, which is widely accepted. Some of the serological relationships, such as *Atropa* and *Withania*, *Physalis* and *Solanum*, and all these with *Lycopersicon*, and possibly also the group of *Datura*, *Lycium*, and *Witheringia*, agree with taxonomic dispositions based on morphological data, but others, such as the position of *Nicotiana*, disagree.

The relationship of *Dunalia* species to *Capsicum* was surprisingly strong and was therefore investigated by a further study including more species of these two genera and also *Iochroma*, which is sometimes merged with *Dunalia*. The numeric data were analyzed by Similarity Ratio and Group Average clustering (Fig. 5) and by Dot Product and Furthest Neighbour (Fig. 6). In both cases *Capsicum annuum* was grouped with *C. baccatum* and then with *C. chacoense*, the several species of *Dunalia* were grouped fairly close to each other and also to *Capsicum*, and the two species of *Iochroma* were separated from the other taxa. *Lycium cestroides* showed a low level of similarity to anything else: the position of *Atropa belladonna* was different in these two analyses.

The greater similarity of the two cultivated species of *Capsicum* (*C. annuum* and *C. baccatum*) than to the wild one (*C. chacoense*) may be significant. The relationship of *Dunalia* to *Capsicum* indicated here is interesting, because although the cultivated peppers are mostly annual herbs, the wild relatives are shrubs. Now that these results have suggested it, the similarity of some species of *Acnistus/Dunalia/Vassobia* to *Capsicum* becomes apparent. These relationships deserve further investigation by sexual or somatic hybridization experiments. The separation of *Iochroma* from *Dunalia* in current taxonomic treatments is supported by these data.

Conclusions

The results presented here illustrate the value of immuno-absorption techniques in serotaxonomy, particularly for comparisons of species and genera that are too distinct to allow biosystematic investigations by hybridization experiments.

The relationships indicated within and between the genera *Capsicum* and *Dunalia* are potentially important for taxonomy and plant breeding, but furthermore they are comparable to relationships of different sections within *Solanum.* This emphasizes that the gigantic genus *Solanum,* which is unified by a few floral characters such as poricidal anthers, comprises an assemblage of very diverse taxa, which could be considered as distinct genera.

Literature Cited

Felsenstein, J. 1982. Numerical methods for inferring evolutionary trees. Quart. Rev. Biol. 57: 379–404.

Jensen, U. & D. E. Fairbrothers (editors). 1983. Nucleic Acids and Proteins in Plant Systematics. Springer-Verlag, Heidelberg and Berlin.

Lester, R. N. 1979. The use of protein characters in the taxonomy of *Solanum* and other Solanaceae. Pp. 285–304 *in* J. G. Hawkes, R. N. Lester & A. D. Skelding (editors), The Biology and Taxonomy of the Solanaceae. Linnean Society Symposium Series No. 7. Academic Press, London and New York.

———, P. A. Roberts & C. Lester. 1983. Analysis of immunotaxonomic data obtained from spur identification and absorption techniques. Pp. 275–300 *in* U. Jensen & D. E. Fairbrothers (editors), Nucleic Acids and Proteins in Plant Systematics. Springer-Verlag, Heidelberg and Berlin.

Whalen, M. D. 1979. Taxonomy of *Solanum* section *Androceras.* Gentes Herb. 11: 359–426.